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14. ABSTRACT

Nerve agent exposure inhibits acetylcholinesterase, leading to increased and prolonged stimulation of acetylcholine receptors. Common consequences of this cholinergic crisis include seizure activity, neuronal damage and behavioral deficits. The paucity of research directed toward the infant/juvenile population has raised concern because of the unique vulnerabilities of children. In the current study, male and female rats exposed to sarin (GB) were evaluated on tests of spatial memory, locomotor activity and vestibular motor function, as well as neuropathology. Similar to our adult model, we found that juvenile rats exposed to GB exhibited deficits in vestibular motor function for up to 1 week and cognitive deficits in the Morris water maze at 3 weeks post-exposure. In addition, extensive neuropathology and spontaneous recurrent seizures (SRS) were observed. The current results demonstrate the vulnerability of a juvenile population to motor impairments, cognitive deficits, neuropathology and SRS following exposure to GB.

15. SUBJECT TERMS
Nerve agent, sarin, development, juvenile, rat

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Introduction

There is limited research on the developmental toxicity associated with exposure to nerve agents. The majority of research has focused on the toxic effects of nerve agents in adult animal models. In the event of a mass casualty situation involving the release of nerve agents like sarin (GB), infants, children and adolescents are likely to be exposed. It is critical to evaluate whether countermeasures that are effective against nerve agents in adult animals will also be efficacious in young animals. However, prior to the evaluation of countermeasures, there is a need to develop standardized models of nerve agent exposure in young animals. To further those efforts, this project was undertaken to characterize the behavioral, physiological and pathological effects of sarin exposure in rats at various time points in their development.

Keywords

Nerve agent, sarin, development, juvenile, rat

Overall Project Summary

Methods

Male and female rats surgically implanted with telemetry receivers (F40-EET; Data Sciences International, St. Paul, MN) received a subcutaneous (sc) injection of saline (SAL) or one of three doses of GB (0.6, 0.8 or 1.0 LD_{50}; LD_{50} = 212 and 233 µg/kg for males and females, respectively) on postnatal day (PND) 42. Toxic signs were continuously observed for 1-2 hr post-exposure, and the rats were weighed daily (Monday-Friday). Rats were evaluated on a battery of behavioral tasks (Table 1) before being perfused on post-exposure day (PED) 32. Brains were removed and sent to FD Neurotechnologies, Inc. (Columbia, MD) for processing. An observer blind to the treatment groups quantified the amount of NeuN-stained cells in brain regions known to be sensitive to nerve agents (amygdala, hippocampus, piriform cortex and thalamus).

Table 1. Behavioral Tasks

Behavioral Task	Cognitive Measure	Post-Exposure Day (PED)
Balance Beam, Runway	Motor coordination, gait	0, 1, 7
Figure-8 Maze	Motor activity	0, 1, 7
Morris Water Maze	Spatial memory and learning	22-25

In addition, a subset of male rats implanted with jugular catheters were exposed to 1.0 LD_{50} GB. Blood was collected at various time points after exposure and analyzed for cardiac troponin (cTnI) levels. At 72 hr post-exposure, the rats were euthanized and their hearts removed for pathology.

Results

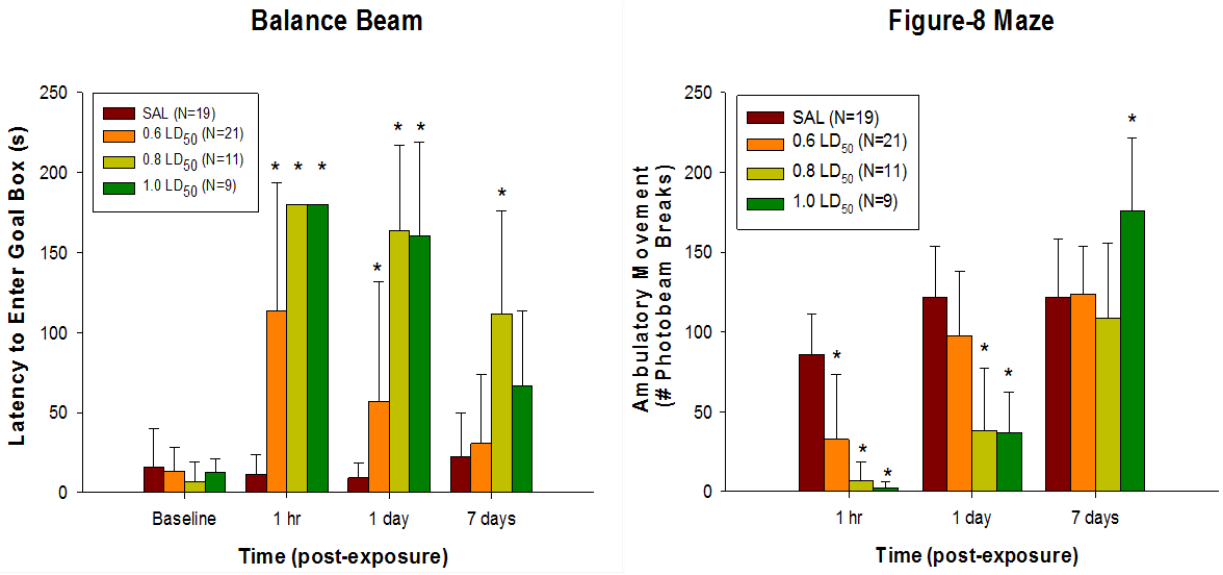

Figure 1. Results showing latency to enter goal box (mean ± SD) on a balance beam task (left), as well as ambulatory movement (mean ± SD) in the Figure-8 maze (right), at 1 hr, 1 and 7 days following sc exposure to GB. Compared to controls, rats exposed to 1.0 LD_{50} GB took significantly (*, p<.05) longer to reach the goal box of the balance beam and made fewer movements in the Figure-8 maze at the 1 hr and 1 day time points.

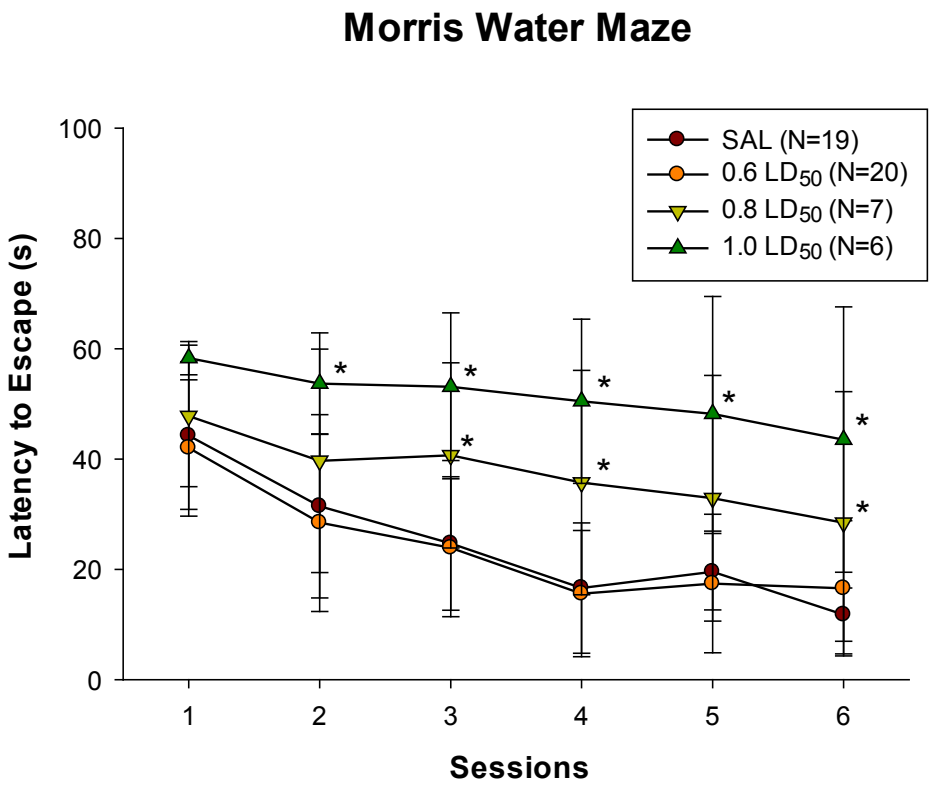

Figure 2. Results showing the latency to escape in the Morris water maze, which was conducted 3 weeks following sc exposure to GB. Each data point represents the mean ± SD. Compared to controls, rats exposed to 0.8 or 1.0 LD_{50} GB on PND 42 were significantly (*, p<.05) impaired on the water maze. In addition, 8 rats had to be rescued from the pool due to convulsions and were unable to complete this task.

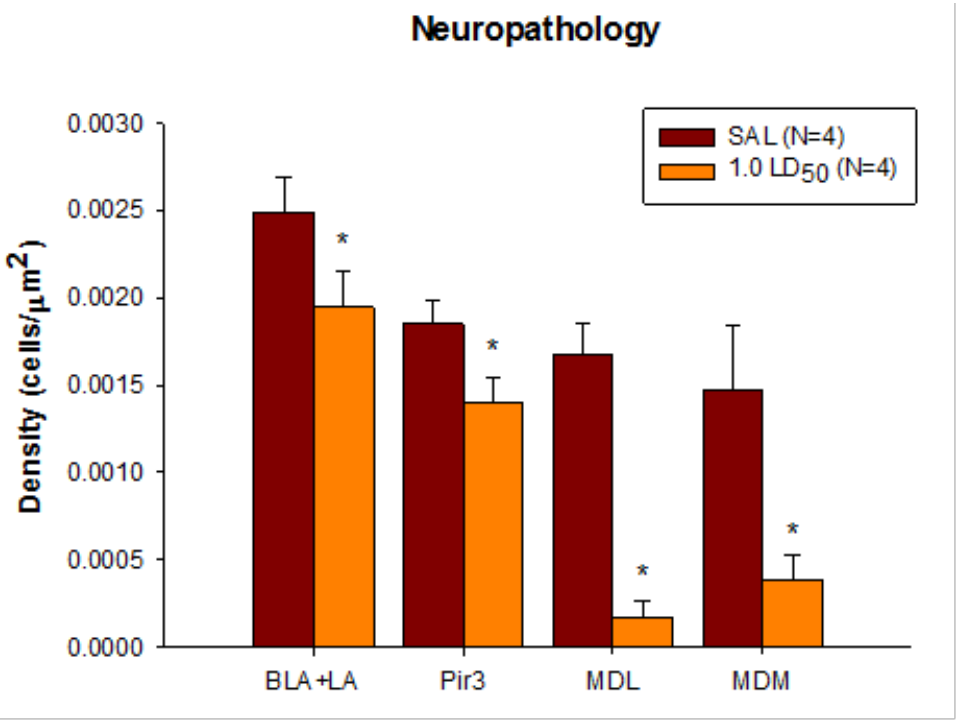

Figure 3. Preliminary results showing the density (mean ± SD) of NeuN-stained cells in various brain regions (basolateral and lateral amygdala, BLA+LA; layer 3 of the piriform cortex, Pir3; mediodorsal thalamic nucleus, lateral part, MDL; and mediodorsal thalamic nucleus, medial part, MDM). The density of new cells was significantly (*, $p<.05$) lower in the brains of GB-exposed rats.

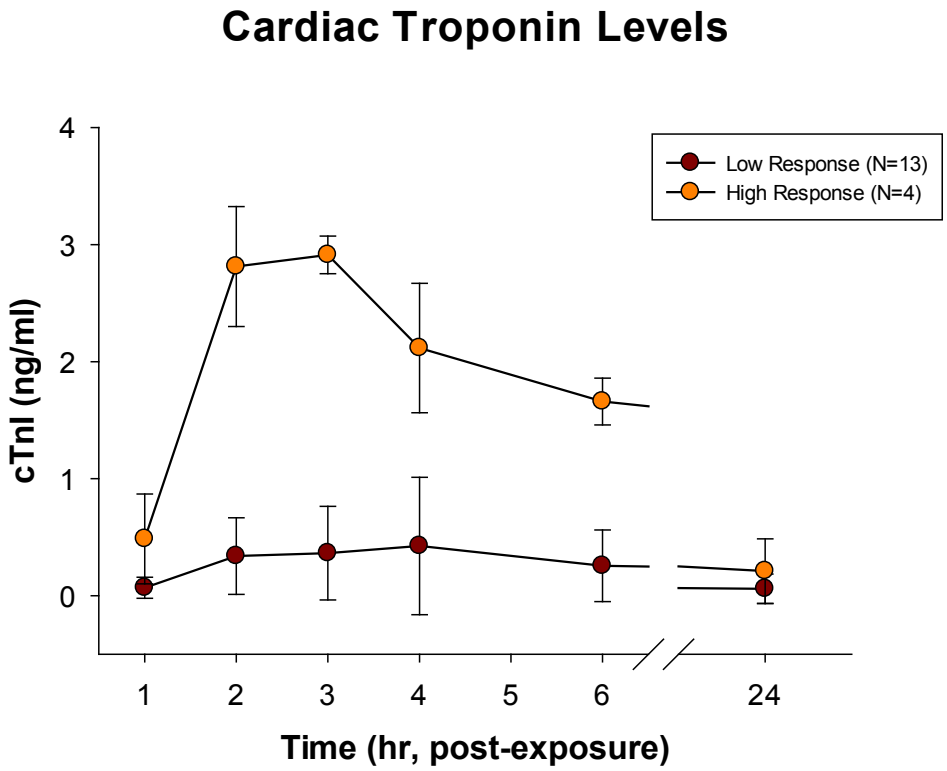

Figure 4. Results showing the levels of cardiac troponin (mean ± SD) in the blood of rats sc exposed to 1.0 LD_{50} GB. Troponin levels peaked within a few (2-4 hr) of exposure before returning to baseline levels at 24 hr. Rats with peak troponin levels greater than 1 ng/ml were classified as high responders, whereas the remaining rats were classified as low responders (peak troponin levels were less than 1 ng/ml).

Key Research Accomplishments

- Characterized the behavioral, physiological and neuropathological effects associated with sc exposure to 1.0 LD_{50} GB in PND 42 rats

Conclusion

Similar to adult models, juvenile rats exposed to 1.0 LD_{50} GB demonstrate motor control deficits and decreased ambulatory movement at 1 day post-exposure, as well as spatial memory impairments at 3 weeks post-exposure. Preliminary data suggests that these rats experience more spontaneous recurrent seizures compared to adult rats (data not shown), which may lead to more extensive neuropathology. In addition, a small percentage of juvenile rats exposed to 1.0 LD_{50} GB have elevated levels of troponin in their blood that may be indicative of myocardial damage. This study shows that nerve agent exposure during puberty results in severe and life-altering consequences in the rat model, which should be an area of further investigation.

Publications, Abstracts, and Presentations

Wright LKM, Miller DB, Muse WT, Emm EJ, Lee RB, Whalley CE and Lumley LA (2014) Younger rats are more susceptible to the lethal effects of sarin than adult rats: 24 h LC50 for whole-body (60 min) exposure. The Toxicologist CD – An Official Journal of the Society of Toxicology 138:581i.

Wright L, Bourne A, Furman A, Stone M, Rossetti F, Lumley L (2014) Behavioral and neuropathological effects associated with subcutaneous exposure to sarin in juvenile rats. Neurotox Teratol 43:93.

Wright LKM, Whalley CE and Lumley LA (2014) Sarin-induced toxicity in young rats: assessment of lethality, behavior, physiology and neuropathology. 19th Biennial Medical Defense Bioscience Review. Hunt Valley, MD.

Inventions, Patents and Licenses

Nothing to report

Reportable Outcomes

Nothing to report

Other Achievements

Nothing to report

References

N/A

Appendices

N/A